

Amendments to the Specification

At the indicated page and line number, please replace the existing paragraphs with the following paragraphs:

(Page 9, line 16 through page 10, line 3)

Figs. 2A, 2B, 2C and 2D. Primary structure analysis of human ABCA2. Fig. 2A: Amino acid sequence (SEQ ID NO: 2) predicted from the full length ABCA2 CDNA. The 12 predicted transmembrane-spanning segments are indicated with a heavy bar below the sequence and the corresponding numerals below. The 21 predicted N-glycosylation sites on the extracellular surface are indicated by "*". Residues comprising the extended ATP-binding cassettes are underlined and indicated by "ATP binding cassette". The highly hydrophobic domain is underlined and labeled as "HHD". The nucleotide sequence was deposited in GenBank under accession number AF 178941. Fig. 2B: Kyle-Doolittle hydrophobicity analysis of ABCA2 protein. The shaded areas above line are hydrophobic and those below are hydrophilic. Analysis was performed in GCG software. Fig. 2C: Schematic showing the predicted topology of ABCA2 in the membrane. The extracellular surface is on the top and the cytoplasmic on the bottom of the figure. The stylized rods on the cytoplasmic loop show N-glycosylation sites. Fig. 2D: Dot-matrix plot of human ABCA2 against itself, with PAM 250 matrix and a window of 8 residues, shows little sequence similarity outside the nucleotide folds.

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Figs. 3A and 3B. Multialignment of the amino acid sequences of the extended nucleotide binding cassettes from the group of ABC1-similar proteins. Clustal-W analysis was performed in Mac Vector software. Shading highlights the conserved residues. Underlined are ATP binding cassette sequence motifs: WA stands for Walker motif A; WB stands for Walker motif B and ATS corresponds to active transport

signature. The reported sequences were extracted from the GenBank database, under the following Accession numbers: ABCA4 (human): NM_000341; ABCA3 (human): NM_001089; ABCA2 (human) AF178941; ABCA1 (human): AAF86276; abca2 (mouse): CAA53531. From top to bottom, Fig. 3A shows SEQ ID NOs: 26, 28, 30, 32, and 34 and Fig. 3B shows SEQ ID NOs: 27, 29, 31, 33, and 35.

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5' RACE using nested-PCR was performed using human brain Marathon-Ready adapter-ligated CDNA as template (Clontech, Palo Alto, CA). The first round of PCR used adapter primer 1 (AP1) 5'-CCATCCTAATACGACTCACTATAGGGC-3' (forward; Sequence I.D. No. 3) and ABCA2 specific 5'-TGAGTTTGTCCACGCAGACAACCAGAG-3' (reverse; Sequence I.D. No. 4); adapter primer 2 (AP2) 5'-ACTCACTATAGGGCTCGAGCGGC-3' (forward; Sequence I.D. No. 5) with ABCA2 specific 5'-CCAGCTCCACTCCCAGGCTTCTG-3' (reverse; Sequence I.D. No. 6). The products were ligated into the pT-Adv plasmid (Clontech, Palo Alto, CA) and ligation extraction products used to transform TOP010' cells. Based on additional 5' sequence obtained from this approach, (clone 35, Fig. 1), the following primers were designed for subsequent 5'RACE: AP1 (forward) and 5'-TGAGTTTGTCCACGCAGACAACCAGAG-3' (reverse; Sequence I.D. No. [[7]] 4); second round PCR used AP2 (forward) and 5'-CCAGCTCCACTCCCAGGCTTCTG-3' (reverse; Sequence I.D. No. [[8]] 6). The longest clone isolated, designated 208 was ~900 bp (Fig. 1).

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To obtain the entire 5'end sequence, we constructed a 5' ABCA2 cDNA library from high quality human brain poly (A)+ RNA (Clontech, Palo Alto, CA) using Marathon cDNA Amplification Kit (Clontech, Palo Alto, CA). Gene specific primer was used for first strand synthesis. Second strand synthesis and creation of blunt ends was followed by DNA ligation with adapters provided with the kit, according to the manufacturer's

protocol. The library was then PCR amplified using AP1 and 5'-CCACTGGGCAGCGAGAAGTTGTC-3' (Sequence I.D. No. [[9]] 7) followed by amplification with nested ABCA2 primer 5'-GAAGCTGGAGTTCTGGCGGATCT-3' (Sequence I.D. No. ~~10~~ 8) and adapter primer, AP2. Reaction products were cloned into pCR-XL-TOPO plasmid (Invitrogen, Carlsbad, CA) and transformed into DH5 α cells. Figure 1 indicates the location of the two longest clones, designated 65 and 119.

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For 3'RACE, we used: 5'CAGACCACACTGGACAATGTGTTCGTG3' (forward; Sequence I.D. No. ~~11~~ 9) and AP1 (reverse); 5'TCATCAGCTTCGAGGAGGAGCGG3' (forward; Sequence I.D. No. ~~12~~ 10) and AP2 (reverse). Only short, 300 bp fragments were isolated (data not shown).

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Full length ABCA2 cDNA was assembled from 4 fragments (A-D). Fragments A and B were obtained by PCR from human brain CDNA library using the following primers:

5'ATAAGCTTGCTGAGGCGGCGGAGCGTGGC3' (Sequence I.D. No. ~~13~~ 11) and 5'CCACTGGGCGAGAAGTTGTC3' (Sequence I.D. No. ~~14~~ 12) for fragment A, and 5'CCTCATTTTCCCCTACAACC3' (Sequence I.D. No. ~~15~~ 13) and 5'ACCTGCTCCATCTTGCTGCTGAACAC, (Sequence I.D. No. ~~16~~ 14) for fragment B. Fragment C was directly obtained by restriction digest of KIA1065 clone (kindly provided by Dr Takahiro Nagase from Kazusa DNA Research Institute). Fragment D was obtained by PCR from KIA1065 clone using 5'CAGCGGCGGCAACAAGCGGAA3' (Sequence I.D. No. ~~17~~ 15) and 5'GGTGAATTCGGCAGGCACTGGGGGACTTGT3' (Sequence I.D. No. ~~18~~ 16) primers. PCR products were initially cloned into pCR-XL-TOPO cloning vector. Fragment A was excised by Hind III and Sal I digest and subcloned into pCR-XL-TOPO clone containing fragment B. Fragment D was excised by Kpn I and EcoR I digestion and

subcloned into corresponding sites of (A+B) pCR-XL-TOPO construct. Finally fragment C was cloned into Kpn I site of (A+B+D) pCR-XL-TOPO clone. The full length ABCA2 cDNA was excised from pCR-XL-TOPO-ABCA2 by Hind III and EcoR I digestion and subcloned into corresponding sites of pcDNA (3.1+) vector (Invitrogen, Carlsbad, CA). That clone has been designated as pcDNA (3.1+)-ABCA2.

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pEGFP-ABCA2 clone was constructed in the following way: start codon of ABCA2 was modified using PCR (primers used were 5'TAGTACTCCTTGGGCTTCCTGCACCAGC3' (Sequence I.D. No. ~~19~~ 17) and 5'CCAGGGCAGATGAGGGACCAAAGA3' (Sequence I.D. No. ~~20~~ 18)), and resulting clone inserted into ScaI and EcoRI sites of pEGFP-C3 vector (Clontech, Palo Alto, CA). All PCR products were verified by double-stranded DNA sequencing.

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5' RACE was used to map the start site of the ABCA2 transcript. Reverse transcription of total brain RNA (Clontech, Palo Alto) was performed using antisense gene specific primer, 5'CATCCAGCAGGTCCCCAGAAGC 3' (Sequence I.D. No. ~~21~~ 19) and was followed by RNase H treatment. The first strand synthesis product was subjected to dC tailing reactions with terminal deoxynucleotidyl transferase. The first round of PCR amplification was then performed using 5' RACE anchor primer 5'GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG3' (Sequence I.D. No. ~~22~~ 20) and gene-specific antisense primer 5'AAACAGGTGCCCTTCCTCCACCAC3' (Sequence I.D. No. ~~23~~ 21). A second round of PCR amplification was performed with universal amplification primer 5'GGCCACGCGTCGACTAGTAC3' (Sequence I.D. No. ~~24~~ 22) and gene specific antisense primer 5'ACAGCGATTGCATGACAGGCAG3' (Sequence I.D. No. ~~25~~ 23). A single ~ 300 bp product was obtained, and after purification it was

cloned and sequenced.

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A multiple human tissue poly(A)+RNA dot blot (MTE Array, Clontech, Palo Alto, CA), and Multiple Tissue Northern Blot (MTN, Clontech, Palo, Alto, CA) were hybridized with a 870 bp probe that was PCR amplified using the following primers: 5'AGGGAGCTGGCTACACCGACG 3' (forward; Sequence I.D. No. ~~26~~ 24) and 5'CGCCTGTGACCACCCGCATCT 3' (reverse; Sequence I. D. No. ~~27~~ 25). Portions were biotin-labeled (for MTE Array screen) or ³²P radioactive labeled (for MTN screen) according to the random primer labeling method. Non-radioactive detection was performed with North 2 South Chemiluminescent Detection system (Pierce, Rockford, IL). The signal intensities were quantified with NIH Image software.

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A number of potential phosphorylation sites were also apparent, including, protein kinase C, casein kinase, tyrosine kinase and cAMP dependent-protein kinase. Of particular interest to our earlier observation of the potential role of ABCA2 in the transport of steroids (Laing et al., 1998), a lipocalin signature (GQSRKLDGGWLKV; SEQ ID NO: 36) was identified at position 1424 within the putative regulatory domain and close to the *HHD*. This motif is characteristic of small lipocalin proteins that transport lipids, steroids, bilins and retinoids (Flower, 1996).